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Memorandum

Date January 24, 1992

From Associate Division Director for Chemistry and Toxicology,
Division of Clinical Laboratory Devices, Office of Device
Evaluation, Center for Devices and Radiological Health

Subject Review Criteria for Cyclosporine PMAs

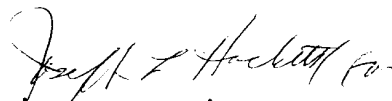
To Interested Manufacturers

We have developed a draft document entitled, "Guidance Criteria for Cyclosporine PMAs." Since the document lists items we will be reviewing, it is intended to assist manufacturers in the preparation of marketing applications for these types of devices.

Since this area of in vitro diagnostics is expanding in the clinical laboratory, we are soliciting your ideas, comments, and suggestions regarding the attached review criteria. We appreciate receiving your remarks because the document will be updated annually.

Please address comments to:

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Attachment

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Guidance Criteria for Cyclosporine PMAs

This is a flexible document representing the current major concerns and suggestions regarding PMA submissions for Cyclosporine Assays. It is based on 1) current basic science, 2) clinical experience, and 3) previous submissions by manufacturers to the FDA. As advances are made in science and medicine, these criteria will be re-evaluated and revised as necessary to accommodate new knowledge.

1. Device Description

Cyclosporine A (CyA) is a natural cyclic undecapeptide isolated from *Tolypocladium Inflatum* Gams (1,2). Several structurally related peptides have been isolated from the same fungus. They include Cyclosporine C, D, and G. Several methodologies have been developed for monitoring CyA therapy. They include FPIA, RIA, and HPLC (3,4). Research studies of CyA immunosuppressive activity and toxicity have employed fast atom bombardment mass spectroscopy (FAB/MS) and nuclear magnetic resonance (NMR). FPIA and first generation RIA's used polyclonal antibodies which exhibit high cross reactivity with circulating metabolites and values obtained exceeded those with HPLC. However, specific and non-specific monoclonal antibodies have been developed (5). The specific monoclonal antibody measures parent cyclosporine and produces similar values to those of HPLC when measuring cyclosporine. The specific monoclonal

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antibody has minimal cross reactivity to metabolites. The non-specific monoclonal antibody measures patient cyclosporine and cross reacts with metabolites of cyclosporine. The non-specific monoclonal antibody produces results similar to the first generation polyclonal antibody.

Nonselective assays measure sample analytes competing with 3H, 125I, or fluorescein tracers for binding to antibodies in polyclonal antisera or a specific murine monoclonal antibody. Most of the available information on cyclosporine and its metabolite levels has been obtained with a radioimmunoassay in which the tracer was 3H and the antibody a polyclonal sheep antiserum. In renal transplant patients the trough cyclosporine values determined with this assay were 40% higher than those measured by HPLC for parent CyA. However, in patients with disturbed cyclosporine metabolism and excretion, such as liver transplant recipients, the results of the two methods deviated by as much as 1500% (6).

11. Background

It is recognized that clinical response does not correlate well with the administered dose. The optimal range of concentrations of the parent drug in the blood, the range required for immunosuppression but which produce the least degree of toxicity, is narrow (7).

There is great variation in the way therapy with CyA is handled at various medical centers in the U.S.A., Canada, and Europe (4). The major differences are as follows:

1. Dosing schedules.
2. Biological fluid selected for analysis.
3. Conditions under which blood samples are processed.
4. Methods of analysis.
5. Therapeutic ranges used for dosage adjustments.
6. Choice of coadministered immunosuppressive drugs.

As experience with CyA monitoring has accumulated, the early post-transplant target CyA concentration ranges have narrowed and they are further lowered at one to six months post-transplant. However, differences in measured CyA concentrations ascribable to differences in assay methods and sample matrix are a major problem in the interpretation of currently available data.

Although adverse clinical events tend to correlate with trough values, almost half of renal transplant recipients have drug levels that are inconsistent with published target values for their renal status, suggesting considerable overlap in target values for ineffective, effective, and toxic trough concentrations (8). This may be due in part to the fact that the contributions to nonspecific measurement of inactive metabolites or measurement of partially active metabolites such as M17 are not understood.

111. Analytical Performance Considerations

A. Pharmacokinetic Studies

These studies should consist of a series of specimens (whole blood, serum, or plasma) taken from healthy volunteers immediately after receiving single oral doses of cyclosporine. It is recommended that heparinized blood be collected immediately prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 96 hours after each dose. These samples should be analyzed to follow the individual pharmacokinetic profile of each participant, and to assess the performance of the assay in comparison to previously-approved assays.

B. Method Comparison Studies

Method comparison studies should be conducted against previously-approved assays (Bio-Rad and Abbott Assays). A minimum of 150 different patient specimens from each matrix (whole blood, plasma, or serum) are suggested. The studies should consist of at least 50 samples from each patient group (kidney, heart, and liver, etc.). Patients should be receiving cyclosporine daily either by I.V. infusion or orally per os (OD, BID, or TID). It is recommended that method comparison data be collected from at least two sites. The methods used to evaluate clinical results should include: 1) method comparisons, including linear

regressions, correlation analyses, data summaries, and nested analyses, 2) covariance analysis, and 3) concentration distribution and mean values of cyclosporine in samples with normal creatinine levels.

C. Precision Studies

1. Within Assay Variability

Three samples of whole blood, serum and plasma should be replicated a number of times (N=20) within the same run. Three concentrations (low, medium, and high) of Cyclosporine A should be analyzed for each specimen type. Samples are prepared by adding Cyclosporine A to normal cyclosporine-free whole blood, serum, and plasma to concentrations corresponding to low, medium and high levels. Within assay variability should be calculated using a coefficient of variation. Coefficient of variation (C.V.) is defined as the standard deviation of the samples divided by the sample mean times 100.

2. Between Assay Variability

Three samples of whole blood, serum and plasma should be run in duplicate over a number of days (N=20). Three concentration (low, medium, and high) of Cyclosporine A should be analyzed for each specimen type (whole blood, serum, or plasma). Follow the protocol for processing samples and analyzing results outlined under item 1 (within assay precision).

3. Between Lab Variability

At least three centers should be given the same specimens consisting of all specimen types (whole blood, serum, and plasma). At least 20 specimens for each specimen type (whole blood, serum, or plasma) are recommended. Within-run, between-run, and inter-laboratory precision should be determined.

D. Recovery

Recovery should be determined by adding known amounts of cyclosporine to samples of whole blood, plasma, or serum to obtain low, intermediate, and high concentrations. The samples are analyzed and the percent recovery is calculated by subtracting the amount of cyclosporine originally present in the samples from the amount measured by analysis. Percent recovery is calculated by dividing this result by the amount added and multiplying by 100.

E. Specificity

Cross reactivity with metabolites should be evaluated. If desirable, percent cross reactivity for radioimmunoassays can be defined as the ratio of Cyclosporine A concentration at 50% B/Bo binding divided by the concentration of metabolite at 50% B/Bo.

F. Interference

Interference studies should be conducted to determine if routinely co-administered drugs will interfere. All drugs and physiological test subjects should be evaluated using samples in the matrix for which the device is intended. Numerous factors affect cyclosporine concentrations in blood. They include gastrointestinal dysfunction, liver disease, food, lipoprotein profile, hematocrit, age, and other drugs. A discussion of these factors is appropriate.

IV. Clinical Studies

Clinical performance may be evaluated in collaboration with several clinical investigators. The goal of the investigators should be to assess the performance of the device in clinical settings. It is recommended that at least 100 patient samples be included for each matrix and specimen type. Objectives of the study may include the following:

1. To assess the performance in comparison to other approved assays.
2. To evaluate the relationship between the administration of cyclosporine and blood levels of cyclosporine by following a number of kidney, heart, etc. transplant recipients over at least a 6 month period.

3. To assess average cyclosporine blood levels in patient samples having normal creatinine values in multiple settings. It may be useful to describe the percent of observations with cyclosporine values falling in specific 50 ng/ml ranges for each transplant/sample group. The average and median cyclosporine levels for all groups of patients should be described.

V. Suggested Therapeutic Levels

No firm therapeutic range exists for serum/plasma or whole blood. Some authorities believe the proper use of cyclosporine monitoring is to measure trends. There is wide variation in reported therapeutic factors. They include specimen choice, measurement method, criteria for diagnosing rejection and nephrotoxicity in the case of renal transplants, and transplant type. a number of factors will cause different requirements for optimal levels of cyclosporine. Discussion of expected values should include a consideration of these elements.

1. The clinical state of the patient.
2. Individual differences in sensitivity to immunosuppressive and nephrotoxic effects of CyA.
3. The type of transplant.

4. Individual values cannot be used as the sole indicator for making changes in the treatment regimen.
5. Each patient should be thoroughly evaluated clinically before each treatment adjustments are made.
6. Each user must establish their own range based on clinical experience.

The following statement is recommended following the box labeling:

Caution: The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, coadministration of other immunosuppressants, type of transplant, time post transplant, and a number of other factors will cause different requirements for optimal blood levels of cyclosporine. Individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made and each user must establish his or her ranges based on clinical experience.

V11. Quality Control

Whole blood assays require whole blood controls. It is recommended that the device contain matrix-specific quality control material. The

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concentrations of analyte should be in the normal and abnormal range, corresponding to the concentrations which are critical in the medical interpretation of the test result. It is recommended that at least two controls (one normal and one abnormal) be processed with each run. Store controls and standards in the same manner. Establish QC criteria tailored to the methodology.

The following criteria have been recommended for RIA:

- A. Minimum correlation coefficient for the log-logit linear regression of the standard curve should be >0.980 .
- B. The percent-bound for the zero standard should be within a range of 35% to 60% and the nonspecific binding must not exceed 10%.
- C. The CV for replicates of each standard and control must not exceed 10%.
- D. Controls must be acceptable by the criteria of Westgard et al (12).
- E. Differences between duplicate patient results must not exceed 10% of the mean value of the two.

The following criteria has been recommended for HPLC/TDX:

- A. Controls must be acceptable by the criteria of Westgard et al.
- B. The coefficient of variation for the controls must not exceed 10%.

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VII. Limitations

Include a statement of known limitations of the procedure. State known extrinsic factors or interfering substances affecting results. A number of drug interactions with CyA appear to be mediated at the metabolic level. Additionally, various factors affect cyclosporine absorption (liver disease), distribution (hematocrit), and elimination (age, drugs). These factors should be addressed in the labeling. If specimens cannot be repeatedly frozen and thawed or if time limits apply to certain procedure steps, it may be appropriate to emphasize this information.

Note: Clinically significant drug interactions occur with concomitant drug therapy.

VIII. Labeling

Labeling should conform to 21 CFR 809.10. Sections to be discussed are:

1. Intended Use.
2. Summary and Explanation of the Test.
3. Method Description.
4. Reagent Composition, Preparation, and Storage.
5. Indication of Possible Deterioration of Kit Reagents.
6. Specimen Requirements.
7. Materials Required.
8. Assay Procedure.

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9. Procedural Comments.
10. Standardization and Quality Control.
11. Limitations.
12. Expected Values.
13. Drug Interactions.
14. Performance Characteristics.
 - A. Precision
 - B. Parallelism
 - C. Recovery
 - D. Sensitivity
 - E. Specificity
 - F. Interference
 - G. Comparison Studies
15. Clinical Studies

IX. Special Considerations

1. Interference studies should be done on patients with bilirubin retention (liver damage).
2. The submission should contain information on false positive rates.
3. Recovery should not be calculated by subtracting an average base value for negative samples from the determined concentrations.
4. The submission and the proposed package insert should contain comparison data against previously-approved assays.
5. The submission should provide an explanation for why serum and plasma values are desirable when whole blood is the matrix of choice.

6. The analysis of covariance should explain why the model was chosen to find the magnitude of variances that cannot be found directly.
7. The firm should explain why the performance characteristics are acceptable.

X. Metabolites

Cyclosporine is extensively metabolized in humans and in animals. More than 90% of the administered intravenous dose of CyA is excreted as metabolites in bile. Large concentrations of metabolite 17 and 1 are present in the blood and renal tissue of kidney-transplant patients. The clinical significance of the contributions of the 10-17 metabolites to immunosuppression or toxicity and whether or not to monitor them in additions to CyA requires further study. The overall contributions of CyA metabolites to immunosuppressive activity in vitro still remains to be determined. A new short hand designation of CyA metabolite had been proposed which is based on the position of oxidation rather than the previous designations which are based on HPLC patterns (11). M1 (the old M17) has been found to be the most immunosuppressive of all CyA metabolites and is present in concentrations exceeding those of CyA in the blood of kidney-, liver-, and heart-transplant patients. The AACC Task Force on CyA Monitoring recommends that CyA be monitored in whole blood with an assay specific for the parent compound, and that if any metabolites were shown to be active, specific assays for them should be

instituted. If specific metabolites are shown to be clinically significant, specific assays have been recommended for their measurement.

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